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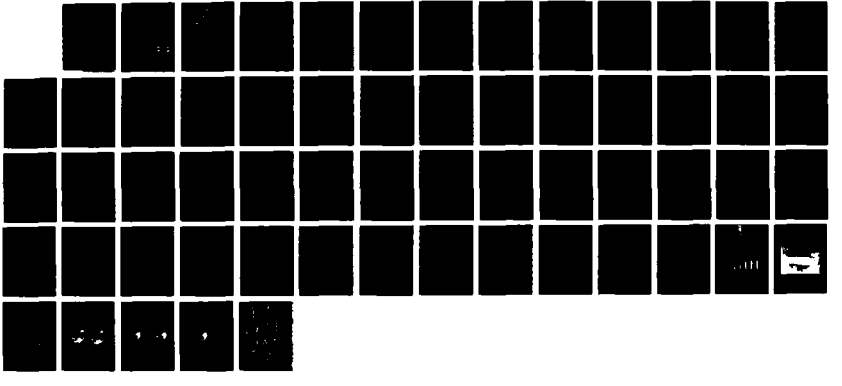

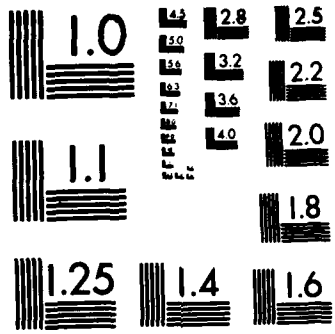

BIOCOMPATIBLE, BIODEGRADABLE POLYMERS FOR USE IN BONE REPAIR

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This chapter presents an overview of biodegradable, synthetic polymers that have been used and that have potential for being used to repair bone defects. Synthesis mechanisms, modes of biodegradation, and properties of the synthetic polymers were discussed. Applications in experimental bony sites were presented.

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BIOCOMPATIBLE, BIODEGRADABLE POLYMERS FOR USE IN BONE REPAIR

I. INTRODUCTION

The need to regenerate bone has inspired the development and application of a vast number of materials to treat skeletal deficiencies. Traditionally, autogenous grafts and allogeneic bone bank preparations are the treatments of choice for skeletal reconstruction. However, owing to the recognized problems with these modalitities,[1-4] certain attractive alternatives have been investigated by our laboratory. Of particular interest to our group are the biocompatible, biodegradable synthetic polymers that may be used as carrier systems for selected bone inducing protein aggregates. The biocompatible, biodegradable polymers offer the advantages of being immunologically privileged, capable of formation into almost any shape, and of degrading naturally and in harmony with new bone formation. There are several excellent reviews on experimental and clinically applied biodegradable polymers.[5-7] Our discussion will be limited to synthetic polymeric materials that either have been investigated for delivering bone inducing proteins or that have potential for such application. Some terms and concepts will be introduced that are germane to this type of bone regenerating system.

II. ATTRIBUTES OF THE POLYMER DELIVERY SYSTEM

A. Terms and Concepts

One of the aims of the skeletal reconstructive surgeon is to restore lost form and function. When osseous regeneration is a goal, the surgeon may use either natural or man-made repair materials.[1] A material that is biocompatible and biodegrades in harmony with newly regenerating bone would be an ideal alternative to autogenous grafts and allogeneic bank bone. A class of synthetic polymers, known as linear polyesters, has been investigated by our laboratory. When certain proteins are combined with the polyesters, the resulting composite appears to have the potential for bone regeneration. The polymers are osteoconductive; that is, they allow for the growth of sprouting capillaries, perivascular tissue, and osteoprogenitor cells.[2] The linear polyesters can function as carrier systems for bone inducing protein aggregates. By definition, bone induction is the process of causing cellular differentiation of osteoblasts from pluripotential mesenchymal cells.[2] Our hypothesis for studying alternatives to traditional bone repair systems is that the polymers will completely biodegrade in harmony with new bone ingrowth. Regeneration will be stimulated by the release of bone inducing protein from the synthetic polymer. The repair system induces bone formation and does not

function as a replacement for deficient osseous tissue; therefore, the new bone will be capable of responding to normal physiologic demands.

Biodegradation of the synthetic bone repair polymer system is an extremely important property; therefore, it would be germane to this discussion to mention some of the possible mechanisms influencing this process. Griffin describes biodegradation as occurring by three probable routes.[8] 1) Direct biodegradation by enzymatic scission of the polymer followed by metabolization of the cleavage products or progressive enzymatic assimilation from the chain terminal. 2) Indirect biodegradation in which compositional diffusion, hydrolysis, and/or oxidative cleavage of the polymer is followed by metabolization of the fragments. 3) Macrobiological degradation in which mechanical attrition by macrophages may be followed by direct and indirect biodegradation. Biodegradation products, then, would include all solubilized compounds that were once a part of the polymer chain: the monomers, oligomers, all leachable polymer additives, and the by-products from their ensuing metabolization. The term bioerosion is sometimes erroneously interchanged with biodegradation. Heller defines bioerosion as the conversion of an initially water insoluble material to a water soluble material by pathways that may or may not involve major chemical degradation.[9]

The biodegradable polyesters have a 25 year history of

safety as suture material. Polyglycolic acid (PGA) was developed by American Cyanamid Co. and has been marketed as Dexon® (Davis and Geck, Inc., Danbury, Connecticut), an absorbable suture; whereas polylactic acid (PLA) was investigated by Du Pont for the same purpose. Since 1970, Dexon® (100% PGA) and Vicryl® (92PGA:8PLA) (Ethicon, Inc., Summerville, New Jersey) biodegradable sutures have been commercially available. The appeal of these linear polyesters is their history of safety, both as intact polymers and because their degradation products are carbon dioxide and water.[7]

B. Microstructure, Morphology, Synthesis

The microstructure of the polymer plays an extremely important role in determining degradation. Like proteins, which are naturally occurring polymers, the synthetics have a primary structure dictated by component monomers and their sequence. This structure determines the conformation that the polymer may assume. Therefore, when the polymer assumes its three-dimensional form, it will have a certain surface topography, which, depending on its geometry and piezoelectric properties, can be recognition sites for the immuno-surveillance system and/or for growth of various living tissues.[10]

The bone regeneration systems that are of most interest to our laboratory consist of the linear polyesters. Linear

polyesters are derived from monomers that have an alcohol and a carboxylic acid functionality. There are three classes of polyesters. One class is unidirectional; it has a hydroxyl terminal and a carboxylate at the opposite terminal. These polyesters are generally produced by the ring-opening polymerizations of lactones; however, some reactions are produced by bacterial fermentation.[11-14] There are numerous reports on lactone polymerizations.[15-26] Lactones are cyclic esters which are produced by the cyclization of a hydroxy-carboxylic acid (Equation 1).

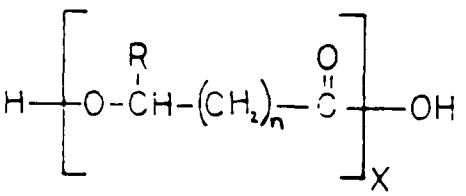
Equation 1

When a hydroxy-carboxylic acid cannot be cyclized because of a limited interatomic distance between the reacting functionalities, (i.e., the alpha-hydroxy carboxylic acids), a dilactone forms by dimerization. Lactide and glycolide are examples of dilactones. These are produced by the catalyzed thermal decomposition of their respective low molecular weight polymers.[27] Table 1 is a list of uni-directional biodegradable polyesters.

TABLE 1

UNI-DIRECTIONAL BIODEGRADABLE POLYESTERS

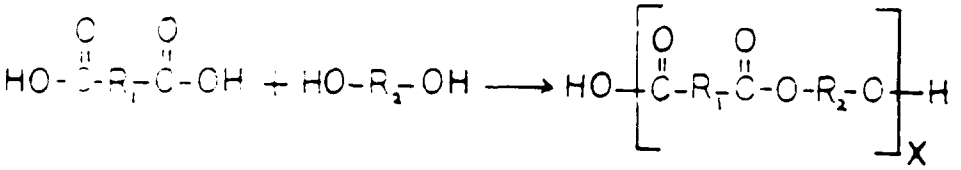
GENERAL FORMULA:



POLYMERS	n	R
HOMO-POLYMERS		
1. Poly(glycolic acid)[16,27,28]	0	H
2. Poly(lactic acid)[19,21,24]	0	CH_3
3. Poly(beta-propiolactone)[16,29-31]	1	H
4. Poly(beta-hydroxybutyric acid)[11,32,33]	1	CH_3
5. Poly(epsilon-caprolactone)[16,34,35]	4	H
CO-POLYMERS		
6. Poly(lactide-co-glycolide)[26,36,37]	0	H,CH_3
7. Poly(beta-hydroxybutyrate-beta-hydroxyvalerate)[38-40]	1	CH_3,CH_2CH_3
8. Poly(glycolide-co-beta-propiolactone)[18]	0,1	H
9. Poly(glycolide-co-gamma-butyrolactone)[18]	0,2	H
10. Poly(glycolide-co-delta-valerolactone)[18]	0,3	H
11. Poly(glycolide-co-epsilon-caprolactone)[16]	0,4	H

The second class of linear polyesters has bi-directionality because they are derived mostly from the polycondensation of dicarboxylate derivatives with diols

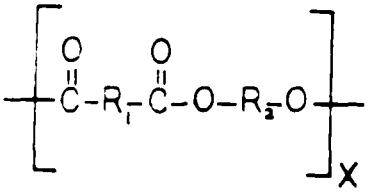
(Equation 2). The terminals in this class can be one of three types, depending on reaction conditions: 1) both hydroxyl, 2) both carboxylate, or 3) one hydroxyl and one carboxylate.

Equation 2 

Only the adducts of oxalate and 1,2-diols are known to give six-membered cyclic compounds that have been polymerized.[41-43] Not many polyesters are derived from the polycondensation route because the polymerization reaction does not yield high molecular weight polymers. Some examples of bi-directional polyesters are shown in Table 2.

TABLE 2

BI-DIRECTIONAL BIODEGRADABLE POLYESTERS

GENERAL FORMULA: 

POLYMER	R_1	R_2
1. Poly(ethylene oxalate)[42,43]	-	$-CH_2CH_2-$
2. Poly(propylene oxalate)[41]	-	$-CH_2CH(CH_3)-$
3. Poly(ethylene terephthalate)[44]	-	$-CH_2CH_2-$
4. Poly(ethylene succinate)[45]	$-CH_2CH_2-$	$-CH_2CH_2-$

By changing the R groups in the monomers, the polymer chemist can derive polyesters that are potentially useful for a variety of biomedical applications. However, the selection of R groups for the monomer is limited by the biocompatibility of the polymer and the toxicity of the monomer and other biodegradation products. It is at this point where polymer development begins, and thus, to date, most biodegradable, biocompatible polymers used for osseous repair are polyesters of known metabolites.

A third class of polyesters is a hybrid. This class is the polyester-ethers, and poly(para-dioxanone) is an example.[46-48] The biodegradable suture PDS® (Ethicon,Inc., Summerville, New Jersey) is a polyester-ether. It is essentially a copolymer of ethylene glycol (a diol) and glycolic acid. It is produced by the ring-opening polymerization of 2-dioxanone which incorporates an ether linkage in the polymer backbone.

C. New Concepts in Polymer Development

The homo- and co-polyesters of lactic and glycolic acids (PLA:PGA) investigated by our group have been shown to be biocompatible[7]. It is plausible that by maintaining the chemical functionalities and not radically deviating from the general structure, biocompatibility will not be lost or compromised.

In addition to biocompatibility, the physical properties of the polyesters are extremely important. It is widely reported in review articles that certain functional groups give the polymer its properties.[49-52] However, none of the articles describe the relationships of these properties to structure, in terms of the nature of the chemical bonds present in the polymer. We have described the linear polyesters used in bone repair as having carboxyl and alkyl groups. These groups give the polymer stiffness and flexiblity. For example, in polyglycolic acid (PGA) (also called poly(glycolide)), there is a 1:1 ratio of carbonyls (C=O) to methylenes ($-CH_2-$). Because there are no pendant groups and because of the strong dipole interactions on the carbonyls of adjacent polymer chains, close packing occurs during crystallization. This results in a highly insoluble polymer. In contrast, polylactic acid (PLA) (also referred to as poly(lactide)) is soluble in common organic solvents because steric hindrance does not allow the chains to pack as closely, even though van der Waals forces allow for close packing of the methyl groups. Although still a crystalline polymer, the lattice energy of poly(lactide) is not as great as that of poly(glycolide), which explains the high melting point of poly(glycolide).[53] When lactide and glycolide are copolymerized at a 50:50 molar ratio, the resulting random copolymer is amorphous and will readily embibe water.[37] As the polymer composition is shifted in favor of one of the

monomers, the resulting blocky regions of each copolymer chain would behave like the respective homopolymer, thereby forming regions of crystallinity. This is confirmed by the observation of Gilding and Reed,[37] whereby lactide-glycolide copolymers having a 25 to 70 mole per cent of glycolide are amorphous. Those copolymers that have mole ratios more or less than 25:70 begin to demonstrate crystallinity. Because crystallinity has a direct affect on the water absorptivity of a polymer, it has, therefore, an indirect affect on its biodegradation. According to Cutright et al., degradation rates of lactide:glycolide copolymers are faster than homopolymers of either polylactide or polyglycolide.[36] Moreover, the copolymers having the highest glycolide content degrade fastest (Table 3). While Cutright et al.[36] did not indicate which stereoisomer of polylactic acid was used in their study, it was likely that it was the amorphous D,L form. The poly (L-lactic acid) degrades slower than the more crystalline polyglycolic acid.[54]

TABLE 3

PLA AND PGA MOLAR RATIOS AND *IN VIVO* DEGRADATION

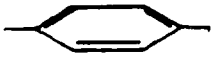
Molar Ratio	Number of Days to Degrade
100PGA	>220
25PLA:75PGA	100
50PLA:50PGA	120
75PLA:25PGA	180
100PLA	220

The methylene to carboxyl ratio and the pendant group type and size are the two principal structural features useful for altering polymer properties. The polymers that have a high methylene to carboxyl ratio have longer degradation times than those polymers whose ratio approaches unity. This is because of the hydrophobic nature of alkyls and the resulting decrease of electrophilic groups. Furthermore, a high methylene to carboxyl ratio results in more saturated (sp^3 hybridized) bonds than unsaturated (sp^2 hybridized) bonds. This allows for freedom of rotation along the polymer chain under periods of stress and strain. For this reason poly(epsilon-caprolactone) degrades slower and is softer than poly(glycolide). Likewise, poly(beta-hydroxybutyrate) degrades slower and is softer than its homologue, poly(lactide).

It is possible to assemble the linear polyesters to conform to desired, specified properties by changing the functionalities of their monomers. Table 4 is a summary of various functionalities with the respective properties that may be introduced to a polymer. It should be noted that some of these functionalities are not necessarily biocompatible.

TABLE 4

POLYMER FUNCTIONALITIES AND PROPERTIES

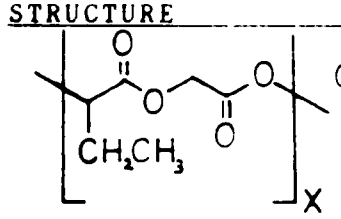
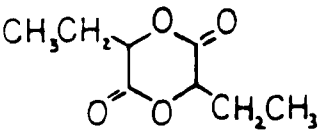
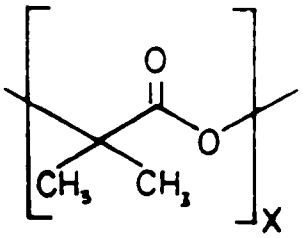
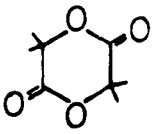
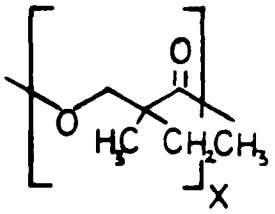
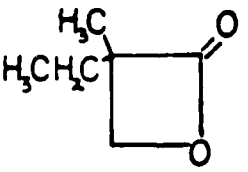
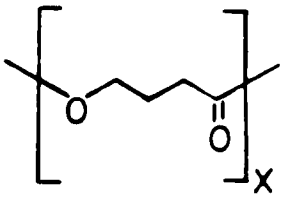
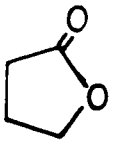
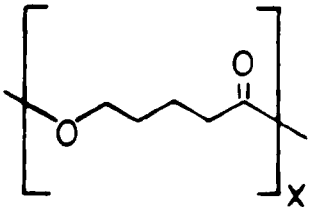
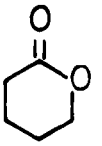
FUNCTIONALY	STRUCTURE	PROPERTIES
1. Aromatic		Hydrophobic rigid
2. Aliphatic	$-(CH_2)_n-$	Hydrophobic flexible
3. Ester	$R-\overset{O}{\overset{\|}{C}}-OR$	Hydrophilic rigid
4. Amide	$R-\overset{O}{\overset{\|}{C}}-\underset{H}{N}-R$	Hydrophilic flexible
5. Ether	R-O-R	Hydrophilic flexible
6. Carbonate	$R-O-\overset{O}{\overset{\|}{C}}-O-R$	Hydrophilic rigid

D. Potential Biodegradable Polymers

For producing high molecular weight polymers, ring-opening polymerization of lactones (Equation 1) is more suitable than polycondensations (Equation 2) of diols with either diacids or hydroxyacids. Lactone rings with three to six carbons within the ring can be used as monomers. This limits the ratio of sp^3 to sp^2 carbons to a range of one to five. However, the pendant group can be present on any of the sp^3 hybridized carbons. There is an almost infinite number of possible monomers that may be used to synthesize polylactone-polyesters (Table 5).

TABLE

MONOMERS FOR POLYLACTONE POLYESTER SYNTHESIS

	STRUCTURE	MONOMER	NOMENCLATURE
1.			Poly (alpha-hydroxy-butyrate)[55] Poly (D,L-ethyl glycolide)[55]
2.			Poly (dimethyl glycolide)[55]
3.			Poly (alpha-methyl-alpha-ethyl-propiolactone)[30]
4.			Poly (γ-butyrolactone)[25,56]
5.			Poly (δ-valerolactone)[57]

III. BIOCOMPATIBILITY OF THE POLYMER BONE IMPLANTS

Evaluation of polymer biocompatibility encompasses an

interaction of the physiological environment on the polymer and effects of the polymer biomaterial on the host environment.[58] The polymer implants that our group uses for bone regeneration are biocompatible because they do not elicit immunologic or chronic inflammatory responses and tissue necrosis does not occur during degradation.

Specifically designing a polymer to have functionalities known to be biocompatible does not preclude testing during development. Preliminary screening of the implant material may be conducted quickly and econcomically *in vitro*. For example, biocompatibility at the tissue-implant interface can be assessed by quantitating the adhesion of radiolabeled chick embryo muscle cells onto polymeric materials.[59] Also, the effect of implants on cellular proliferation and protein synthesis has been studied by adding extracts of biomaterials to 3T3 fibroblasts in the presence of radiolabeled substrates.[60] Materials with a cytotoxic effect caused inhibition of the incorporation of the radiolabeled substrates in the assays. Rice et al. pulverized implant material to a fine powder, which was then added to 3T3 fibroblasts.[61] The effect of the pulverized polymeric material on cellular functions of attachment, viability, and division was monitored by microscopic observation, dye exclusion, and population doubling determinations. Excellent correlation was obtained when *in vitro* results were compared to *in vivo* testing in rats.[62] An *in vivo* assay system has

been developed using rabbits to test the effect of biomaterials on bone formation and resorption.[63] Implant materials also have been tested in the mouse peritoneal cavity and biocompatibility assessment was based on the amount and type of cellular adherence.[64]

Protein adherence to a polymer implant and its interaction with the local tissue affect compatibility. However, as a result of the surgical trauma of implantation, an acute local inflammatory response is mounted. The extent of this response is modulated by local influences, such as the presence of infection, the degree of local tissue vascularization, and the presence of foreign bodies (i.e.,suture material and implants).[65] Damage to vascularized tissue leads to an increase in the permeability of the blood vessels in the vicinity of the injury and blood platelets begin to mediate a thrombotic response in which the plasma protein, fibrinogen, is cleaved and converted into an extravascular mesh work of fibrin and intravascular thrombi (solid plugs of platelets and fibrin). During this process, there is a release of pharmacologically active endogenous chemical substances.[66] Some are chemotactic agents that direct the migration of neutrophils to the site of injury. Neutrophils are the primary phagocytic cell at the site of injury for the first 24 hours. They release chemotactic factors which stimulate other phagocytes, such as monocytes, to migrate to the site of injury. The phagocytic cells

recognize bacteria, cellular debris, and extraneous material by their coating of opsonic proteins. During the process of phagocytosis, lysosomal granules fuse with the phagocytic vacuole and release their hydrolytic and degradative enzymes, thereby either killing or degrading the contents of the vacuole. When the phagocytes cannot completely engulf the extraneous material, proteolytic enzymes are released into the extracellular environment and damage the neighboring cells and matrix. *In vivo* and *in vitro* studies performed with PGA microspheres demonstrated that macrophages phagocytize the spheres.[67] This occurred as early as ten minutes in cell culture, although most of the particles had adhered to the cell surfaces and were surrounded by cytoplasmic processes within two hours and incorporated into cytoplasmic phagolysosomes. However, no evidence of particle degradation was seen even after 48 hours. *In vivo*, polyglycolic acid (PGA) microspheres were present in the lysosomes of monocytes within six hours post-injection. By 24 hours, the Kupfer cells of the liver contained cytoplasmic particles of PGA. After two weeks *in vivo*, residual particles could not be identified.

When the phagocytes are unable to completely degrade a foreign body, fibroblast infiltration and proliferation may be enhanced by the release of platelet derived growth factor(PDGF)[68] and by the presence of proteolytically cleaved fibronectin.[69] Fibroblasts will encapsulate foreign

material, and thickness of the fibrotic capsule is dependent on the biocompatibility of the implant. Biocompatibility is affected by physical and chemical properties and implant degradation products. For example, the physical property of the implant's surface and its relationship with contiguous tissue macromolecules (i.e., proteins) affects adsorption of the proteins onto the polymer surface.[70] Competitive interfacial adsorption of the proteins will occur in order to lower the interfacial free energy or to counter the Donnan equilibrium and other electrical effects.[70,71] Their diffusion to the surface of the implant or into the pores is generally the rate determining step of the adsorption process and is adequately described by first order kinetics.[72] The quantity of adsorbed protein, plateau times, and adsorption rates depend upon the polymer surface . Adsorption of the plasma proteins takes approximately an hour to reach equilibrium.[72,73] Factors which affect this equilibrium may be the extent of hydrogen bonding and hydrophobic interactions between the adsorbate and adsorbent, water structuring at the interface, and the configurational entropy of proteins at the adsorbed sites.[72] This adsorbed layer of protein on the implant then influences the severity of the tissue response.

A major distinction is seen between hydrophilic and hydrophobic implant surfaces. In general, hydrophobic surfaces adsorb plasma proteins irreversibly.[74] It has been

observed that hydrophobic implants elicit minimal soft tissue responses.[74] Conversely, implants with hydrophilic surfaces readily desorb proteins[74] and invoke an abnormal tissue response. Consequently, it has been proposed that there may exist a critical hydrophobic character which an implant surface must possess for essentially irreversible plasma protein adsorption, and that all surfaces with at least this level of hydrophobicity are likely to elicit minimal soft tissue responses.[70]

It is not surprising that PLA, PGA or coploymers of the two, all of which demonstrate hydrophobicity, elicite only a minimal soft tissue response. Herrmann et al. compared PGA suture material to catgut and chromic catgut sutures.[75] Histological evaluation of the implant sites demonstrated that the PGA suture exhibited the least amount of acute inflammation at all time periods. PLA was evaluated by Kulkarni et al.[76] and Cutright and Hunsuck.[77] They determined that PLA elicted a mild inflammatory reaction. At 14 days post-implantation, the PLA suture was engulfed by a fibrous shealth with fibroblasts, histiocytes, scattered lymphocytes, occasional plasma cells, foreign body giant cells, and a dense network of capillaries.[77] By 28 days more plasma cells were present and multinucleated giant cells were seen next to the sutures. At 42 days localized chronic inflammation was evidenced by the presence of foreign-body giant cells and histiocytes which surrounded each strand of

suture material. Several strands of suture had been resorbed while others had been markedly reduced in size. At 56 days the sutures were almost completely resorbed.

Microspheres of PLA, PGA and a copolymer of PLA and PGA were assesed *in vivo*.[78,79] The tissue responses that these polymers elicited were judged as being virtually identical to each other.[78,79] Remnants of the polymer microspheres were present after 63 days.[78] Predictably, small cylindrical implants of 50:50 poly(D,L-lactide-co-glycolide) also elicited a minimal inflammatory response.[7] However, by 35 days post-implantation, a thin rim of histiocytes and multinucleated giant cells lined the implant surface and interstices. This is an observation characteristic of a chronic resorption response to an insoluble, biocompatible polymer.[7] Additional confirmation of PLA biocompatibility was confirmed on the basis of *in vitro* and *in vivo* testing using PGA as the standard noncytotoxic material.[61,62]

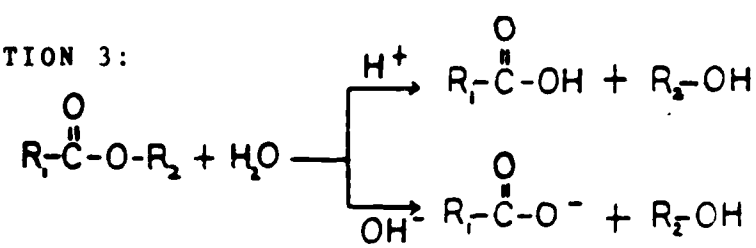
A. Biodegradation of Linear Polyesters

Immediately following implantation, the processes of biodegradation and bioerosion of the polyester implant commences concurrently with the controlled release of its payload of bone inductive protein aggregates. The *in vivo* biodegradation of a polymer may occur by three routes:

direct, indirect, macobiological. Biodegradation may be followed by metabolization of the break down products, although they need not necessarily proceed to the stage where the physical form of the polymer is altered.[6] Conversely, bioerosion will result in a physical loss of the polymer.[6]

The rate of *in vivo* degradation of a polymer will be affected by water, ions, the local pH, and enzymes.[6] Human tissues are approximately 70% water[6] and the diffusion of water into the polymer matrix will initiate hydrolytic chain scission (Equation 3).[6,7]

EQUATION 3:



The ester groups of the polyester based implants are susceptible to hydrolysis. Furthermore, anions and cations and the pH of the local microenvironment may produce a sizable catalytic effect.[6] Hydrophobic polymers such a PLA, PGA and their copolymers, which absorb water with a low diffusivity, will degrade primarily from the surface.[6] The initial hydrolytic chain scission will occur predominantly in the amorphous regions of the polymer. The low molecular weight degradation products will diffuse out of the matrix and form pores.[6,80]

Enzymatic attack of the polymer will occur on the

surface of the implant and progress inward as pores develop.[6,50] *In vitro*[51-53] and *in vivo*[54] studies have demonstrated the involvement of enzymes in the biodegradation of polymers. Williams and Mort reported that ficin, carboxypeptidase A, alpha-chymotrypsin, and clostridiopeptidase A accelerated the *in vitro* rate of degradation of PGA.[51] Williams noted that proteinase K, pronase, and bromelain increased the degradation rate of PGA.[52,53]

Phagocytic cells which attack polyester polymers are a source of hydrolases and oxidases. Neutrophils are the primary phagocytic cell type for the first 24 hours following implantation. They do not contribute significantly to the enzymatic degradation of a polymer implant.[5,84] However, mononuclear macrophages and giant cells have been identified as the predominate suppliers of degradative enzymes.[5,6,67,54] Rough extensions on polymeric implants can be phagocytized and degraded in the phagolysosomal vacuoles of macrophages.[6,67] The rate of this enzymatic degradation passes through a maximum as the phagocytic attack of the implant intensifies and then wanes as the local site is infiltrated by fibroblasts.[6] The rate of degradation decreases again as a collagenous capsule encases the implant.[6,54] A steady state concentration of enzymes, albeit small, may still be maintained next to the implant surface.[6] However, the diffusion of degradation products or

release of a protein from the implant is impaired, as is the replenishment of hydrolases and oxidases. The impaired diffusion, consequently, causes an increase in the local concentration of the polymer degradation products. If they are not biocompatible, they will initiate a toxic response, damaging the local tissue, and possibly impeding the action of inductive proteins.

In addition to the bioenvironmental factors already mentioned (water, ions, pH, enzymes), the mechanical stresses a polymeric implant are subjected to will affect *in vivo* degradation rate. The reaction of the polymer to mechanical stress is directly affected by the bioenvironmental factors which iniate significant alteration in the properties of the polymer.[85] For example, when a stress sigma (σ) is applied, a polymer deforms to a strain, epsilon (ε) (Equation 4). When the stress is removed, the polymer implant attempts to recover its original dimensions based on Young's modulus (Y).[86]

Equation 4

Hooke's Law: sigma (σ) = $Y\varepsilon$

The covalent bonds in polymers are not particularly strong, in contrast to the ionic and metallic bonds found in

ceramics and metals, respectively.[55] Consequently, as the stress applied becomes excessive, the molecular chains of the polyester polymer can be broken, thereby iniating mechanochemical reactions[55] via the production of a pair of free radicals.[37] Subsequent chain scission will decrease Young's modulus. Simultaneous adsorption of low molecular weight species[55] will swell the polymer matrix. Bonds between the amorphous and crystalline regions of the polymer implant will weaken, thereby enhancing deformation and decreasing Young's modulus. Conversely, leaching of plasticizers has the opposite affect of adsorption on Young's modulus. However, the usual result of exposing biomedical implant polymers to physiological fluids is to lower Young's modulus.[85] This increases the rate of deformation of the polyester implant[56] and ultimately increases its rate of degradation.

PLA, PGA, and their copolymers have been shown to be biocompatible[7,75-79] and their degradation products are easily metabolized.[81,88-90] Hydrolysis of PLA will generate lactic acid which is metabolized via the tricaboxylic acid cycle (TCA cycle) and is excreted by the lungs as carbon dioxide and water.[89] Hydrolysis and enzymatic attack of PGA produces glycolic acid monomers.[82,90] The monomeric units of PGA may be excreted in the urine or may be converted to glyoxylate by glycolate oxidase and then further metabolized to glycine by glycine transaminase. The glycine may be used

to synthesize serine, which can enter the TCA cycle via conversion to pyruvate.[90]

IV. BONE REPAIR COMPOSITES OF POLYMERS AND PROTEIN AGGREGATES

While Cutright et al.[91,92] and Getter et al.[93] reported using biodegradable polymers for fracture repair, the first reported use of the polyester PLA:PGA for bone regeneration was made by Nelson et al. .[94] Tibial defects in four groups of rats were treated with 1) PLA:PGA, 2) PLA:PGA plus tricalcium phosphate, 3) tricalcium phosphate, and 4) no treatment. Histological examination after 42 days revealed that bony repair had occurred in the untreated defects and in the tricalcium phosphate group. There was incomplete healing in the PLA:PGA and PLA:PGA plus tricalcium phosphate groups. Hollinger prepared tibial defects in rats and treated them with 50:50 poly (L(+) lactide-co-glycolide.)[90] In another experiment, Hollinger added a protein-acidic phospholipid (diphosphoinositide-lysozyme) to 50:50 poly (D,L-lactide co-glycolide).[95] This composite was used to treat cortico-cancellous wounds in the long bones of 180 adult Walter Reed strain rats. Histomorphometric analyses were performed in both studies and the data indicated: 1) the

protein-acidic phospholipid-copolymer composite produced the most rapid healing rate; 2) elements of osseous repair were in greater abundance in the copolymer treated group than in the untreated controls, and 3) partial degradation of the implants was evident at three days. Because of the promising results with the composite implants used in the rat studies, Hollinger and Schmitz prepared composite osteoconductive implants of 50:50 poly (D,L-lactide-co-glycolide):diphosphoinositide-lyzosyme for treating continuity defects in the mandibles of 25 adult Foxhound dogs.[96] Each dog received bilateral mandibular body defects: one untreated control and one treated with the experimental composite implant. Over the 40 week course of the experiment, the experimental sides produced a greater quantity of trabecular bony volume than did the untreated controls (Figure 1). We hypothesize that the positive bone healing responses encountered in the composite implant experiments may have been due to the development of a unique chemical environment for calcium and phosphate precipitation, nucleation, and subsequent crystal growth.[95] Furthermore, the proteolipid of diphosphoinositide-lyzosyme has been described as being tantamount to a surrogate extracellular matrix vesicle, the structure whose limiting membrane is heavily endowed with a proteolipid component similar to the one used by us.[95,97] Importantly, matrix vesicles are recognized for their extremely important contribution to the process of

calcification.[98,99] The linear polyester component of the composite implant could have served as a matrix, trellis, or foundation for consolidation of the bony reparative elements.[95] The importance of type I collagen for ossification has been reognized and attributed to its geometry and surface charge.[100,101] There may be a similar affect from the linearly arranged PLA:PGA macromolecules comprising the composite implant. Furthermore, the implant functions to distract or obviate initial soft tissue collapse into the skeletal wound. This enables the development of bony regenerative elements from the skeletal envelopes and marrow. In addition, as the PLA:PGA degrades there is a shift in local pH towards increasing acidity. We speculated that such an alteration in the microenvironment could affect calcification inhibitors such as proteoglycans and glycosaminoglycans. Furthermore, at the recipient bed the degrading polymer could act in a beneficial manner to promote release of certain bone inducing protein aggregates (i.e., bone morphogenetic protein, skeletal growth factor).[102] Despite the fact that the composite PLA:PGA and diphosphoinositide-lyzosyme implants appear to be effective bone regenerating materials, they were not satisfactorily evaluated in an unequivocal critically sized skeletal defect.[103] Moreover, the proteolipid diphosphoinositide-lyzosyme is a calcification inducer rather than an ossificiation inducer. Consequently, our laboratory has been

investigating several types of bone inductive protein aggregates that can be combined with a PLA:PGA carrier system. An example of one of these composites was prepared from a bone matrix derivative that was combined with 50:50 poly (D,L-lactide-co-glycolide) in the form of 15.5 mm by 3.5 mm disks for craniotomy repair in adult rabbits.[104] The craniotomies were unequivocal critical sized defects. The composites produced clinical osseous union as early as eight weeks without any evidence of residual PLA:PGA (Figure 2). Because of the complete biotolerance and favorable osteoinductive and osteoconductive attributes of this combination, similar bone inducing derivative proteins and polymer composites were prepared for the treatment of non healing craniotomies in Macaca mulatta (rhesus) non-human primates.[105] While the composites produced bone regeneration, the degree of osseous healing was not as dramatic as in the rabbit experiment (Figure 3). It is conceivable that ascending the phylogenetic tree presents some difficuties to the skeletal reconstructionist that are not encountered in the lower species. It may be necessary, therefore, to produce a more potent bone inducing cocktail for use in regenerating bone in the non-human and human primates. Our group is currently investigating the addition of selected growth factors for augmenting the bone derivative inducing protein aggregates.

V. PLANS

There are significant problems that must be resolved before a reliable bone regenerating composite becomes a standard treatment modality for the skeletal reconstructionist. 1) The tempo of degradation of the biodegradable carrier (i.e., the polyester of 50:50 poly (D,L lactide-co-glycolide)) must be sequenced harmoniously with bone regneration. 2) Release kinetics of the bone regenerating factors from the biodegradable carrier must be balanced temporally with the availability of pluripotential cells. 3) Amino acid sequences of bone regenerating proteins must be identified to allow for laboratory synthesis. 4) The interaction of inducing factors and growth factors must be packaged properly within the biodegradable polymer to mimic Mother Nature. 5) Vascularization at the recipient bed must be accomplished with the implant composite.

Progress recently has been made in our laboratory towards resolving some of the issues plaguing the successful development of a biocompatible, biodegradable bone repair material. One important step that we have taken is in the design of the implant. We have recognized that a monolithically designed implant composite will not satisfactorily exploit the potential of the host marrow

(Figure 4). A Multiphase System Implant has been designed to prevent soft tissue collapse into continuity gaps (Figures 5A,B). This type of implant will allow for immediate marrow cell interaction in concert with pluripotential cells of the bony envelopes. Sequenced release of bone inducing protein aggregates from biodegradable PLA:PGA envelopes will occur within the lumen of a hollow PLA:PGA tube. The "macrospheres" will have envelopes of different PLA to PGA molar ratios for a "timed release" affect. Moreover, the physical properties of the wall of the hollow tube will militate against biodegradation and attendant soft tissue collapse until the bony fragment ends have united with osseous tissue.[106]

VI. CONCLUSION

Our ultimate goal is to develop a completely laboratory synthesized bone regeneration implant. The new bony tissue will be enduring and assure for the return of form and function. Moreover, it will be bone that can respond to normal physiologic requirements. Such a lofty endeavor requires a highly structured, disciplined and organized multidisciplinary approach that involves biochemistry, physiology, surgery, toxicology, biomaterials technology, and

polymer chemistry. Our disciplines still have an arduous journey ahead before accomplishment of our goal.

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LEGENDS

1. Figure 1: Means and standard deviations (across five animals after taking the mean of duplicate measurements) of trabecular bone development in implant treated and untreated defects. The copolymer-PL implants were 50,50 poly (D,L-lactide co-glycolide) plus diphosphoinositide-lysozyme.

2. Figure 2: Photomicrograph of treated 15 mm craniotomy in rabbit parietal bones at eight weeks. The implant consisted of 50,50 poly (D,L-lactide co-glycolide) plus a bone matrix derivative. (Arrows delimit host bone. Goldner trichrome stain. Macrophotograph magnified three times.)

3. Figure 3: The trabecular bony volume that developed in craniotomies in rhesus non human primates at three and six months. The quantity of bone was greatest at three months for PMCB (particulate cortical bone marrow), followed by AA CO (50,50 poly (D,L-lactide co-glycolide) plus bone matrix derivative), BMP CO (50,50 poly (D,L-lactide co-glycolide) with bone morphogenetic protein), and C (untreated controls). The six month groups displayed the same trends.

4. Figure 4: A biodegradable alloplastic implant block used to restore mandibular continuity did not allow for sufficient new bone development across the gap. The Monolithic design resulted in soft tissue collapse into the wound bed.

5. Figures 5A and B: A Multiphase System Implant for optimization of the participation of the bone marrow compartment and soft tissue bony envelopes for regenerating bone across continuity gaps.

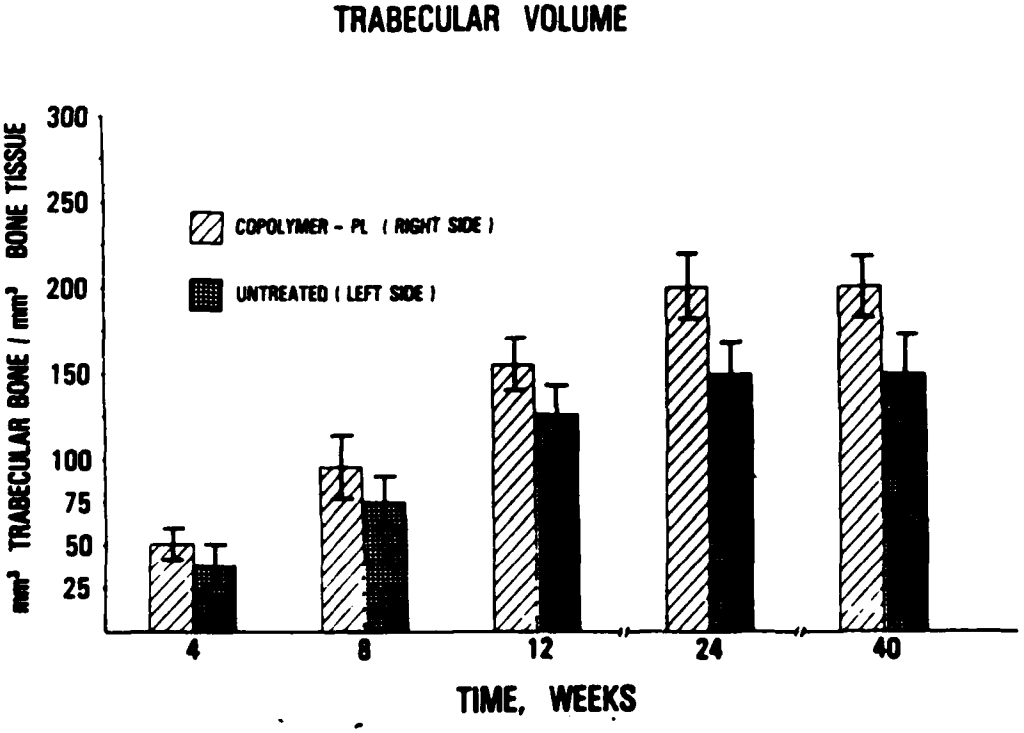

TRABECULAR VOLUME
mm³ TRABECULAR BONE / mm³ BONE TISSUE
300
250
200
150
100
75
50
25
COPOLYMER - PL (RIGHT SIDE)
UNTREATED (LEFT SIDE)
4
8
12
24
40
TIME, WEEKS

Figure 1

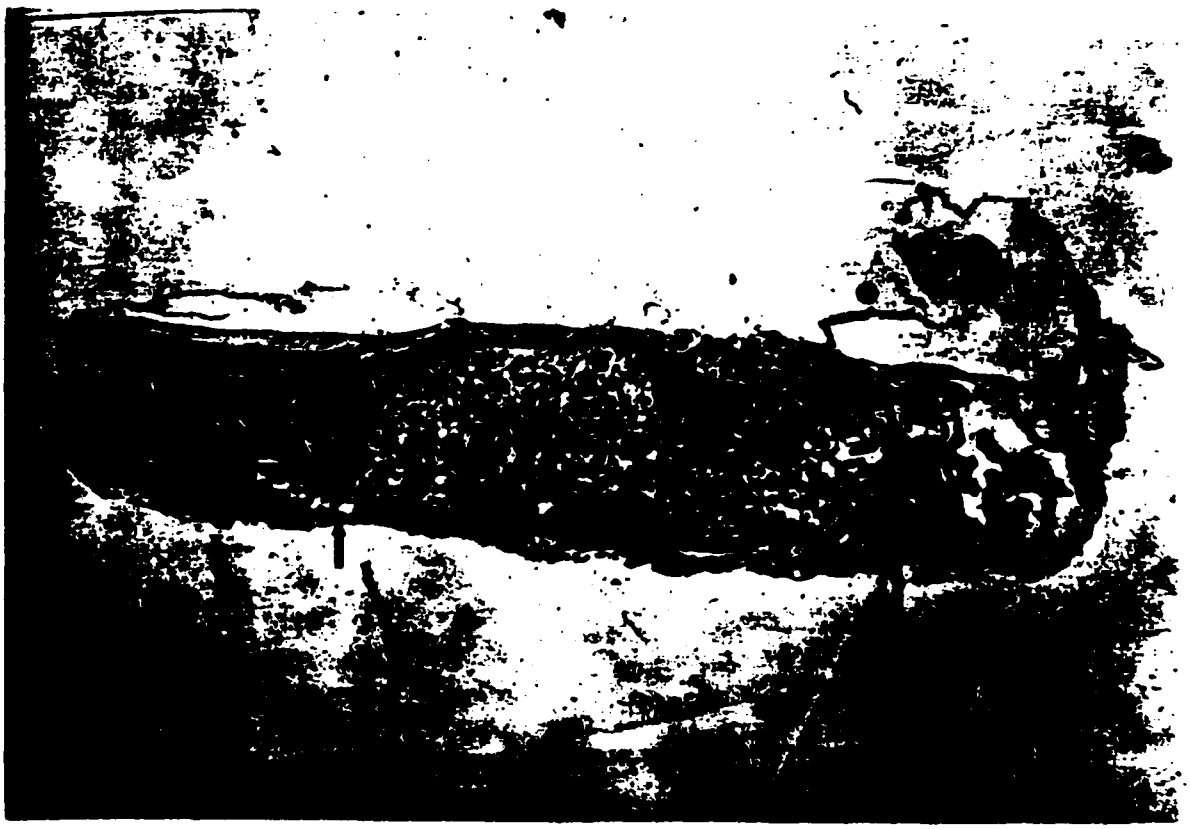


Figure 2

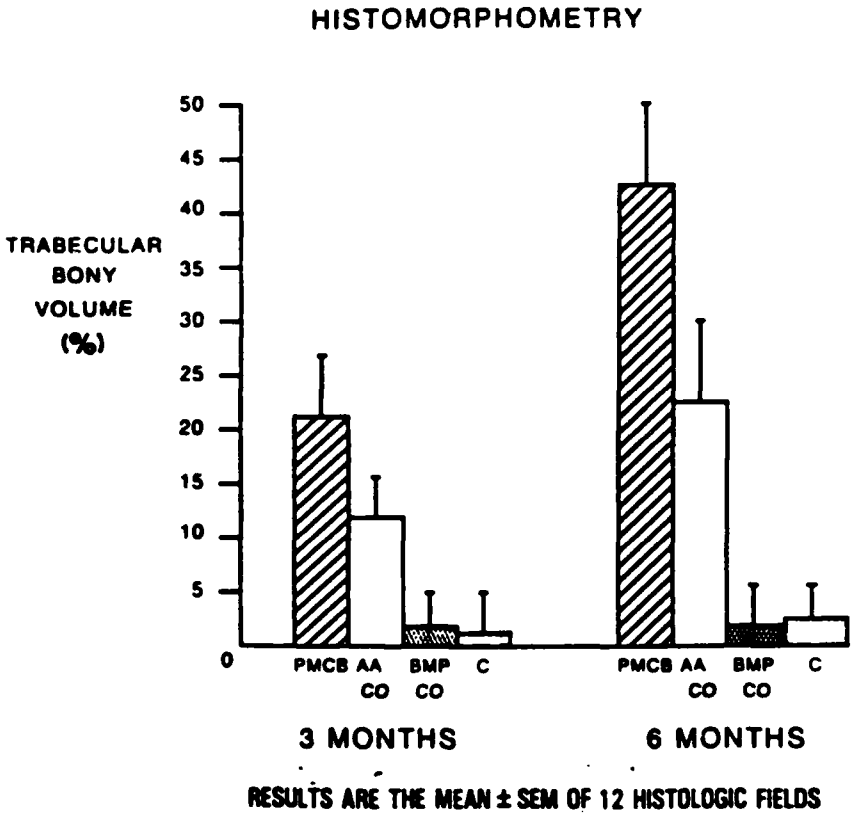
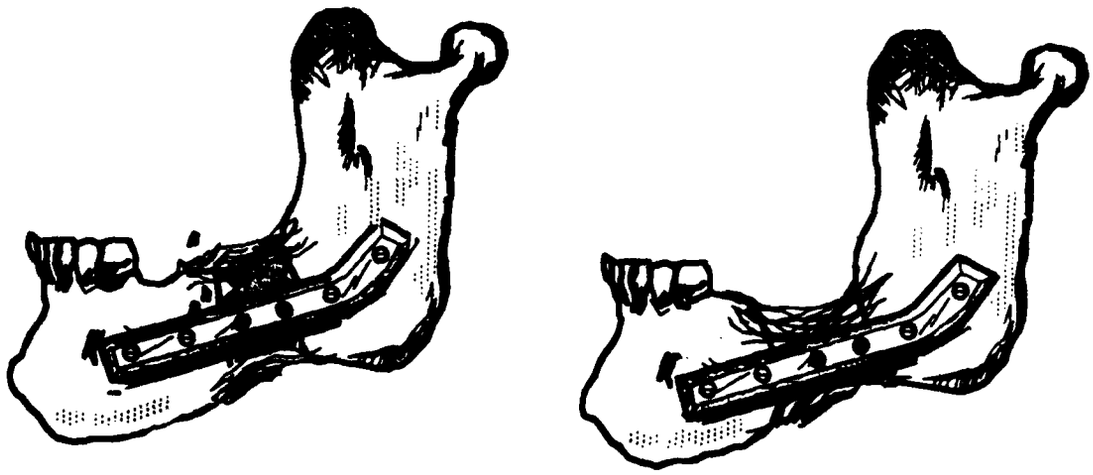

HISTOMORPHOMETRY
TRABECULAR BONY VOLUME (%)
50
45
40
35
30
25
20
15
10
5
0
PMCB
AA CO
BMP CO
C
PMCB
AA CO
BMP CO
C
3 MONTHS
6 MONTHS
RESULTS ARE THE MEAN ± SEM OF 12 HISTOLOGIC FIELDS

Figure 3

MONOLITHIC IMPLANT



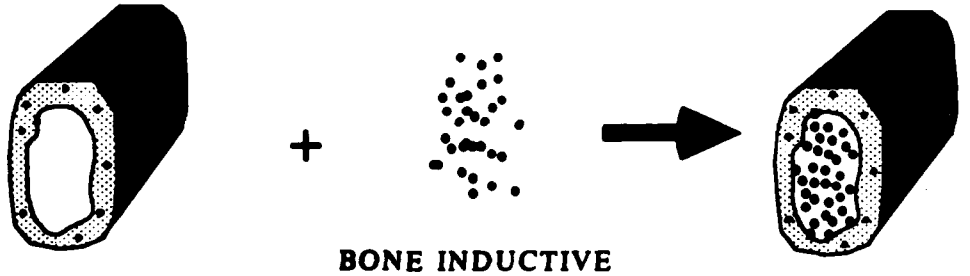
SOFT TISSUE COLLAPSE

FIBROUS UNION

Figure 4

MULTIPHASE SYSTEM IMPLANT

- NO SOFT TISSUE COLLAPSE
- SEQUENCED INDUCTION OF NEW BONE



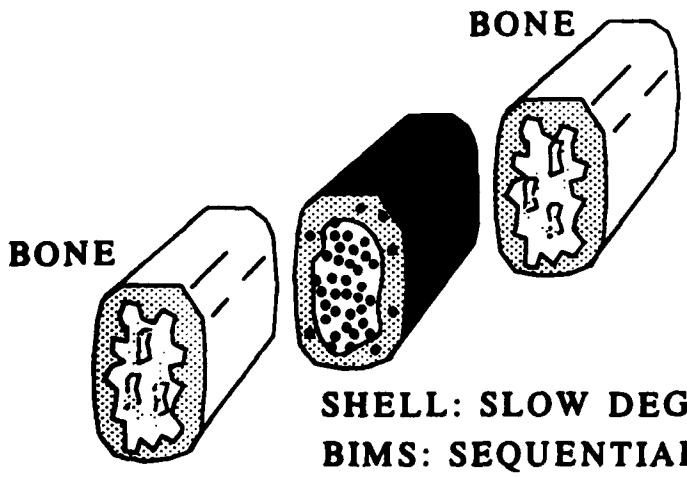
SHELL CONTAINS BONE INDUCTIVE COMPONENT

BONE INDUCTIVE MICROSPHERES

— BIMS —

Figure 5 A

MULTIPHASE SYSTEM IMPLANT



SHELL: SLOW DEGRADATION
BIMS: SEQUENTIAL DEGRADATION

Figure 5 B

END

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